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Molecular cloning and sequence of partial cDNA for interferon-induced (2′-5′)oligo(A) synthetase mRNA from human cells

(recombinant DNA/hybridization-translation/*Xenopus* oocytes)

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ABSTRACT **By using a translation assay in oocytes, a 17S RNA fraction coding for the interferon-induced (2′-5′)oligo(A) synthetase was purified from human cells. A cDNA library was prepared by cloning in *Escherichia coli* plasmid pBR322 and screened by positive hybridization-translation in oocytes. A cDNA clone corresponding to the (2′-5′)oligo(A) synthetase mRNA was identified. In SV80 cells, this E cDNA recognizes three RNAs of 1.65, 1.85, and 3.6 kilobases, which are present only after interferon treatment of the cells. In Namalva cells, mainly one RNA of 1.8 kilobases is seen.**

All interferons (IFNs) induce in cells the synthesis of a certain number of proteins and their mRNAs (1–3). These induced proteins include translation regulatory enzymes (1–3), surface antigens (4–6), and unidentified proteins (7–9) and are probably involved in the complex antiviral, antimitogenic, and immunoregulatory effects of IFNs. Among the enzymes induced by IFNs, the (2′-5′)oligo(A) synthetase has been best studied. This enzyme binds double-stranded (ds) RNA and polymerizes ATP into ppp(A2′p)nA dimers, trimers, and longer oligomers (10), which activate the latent RNase F (11–13), thereby inhibiting protein synthesis. The (2′-5′)oligo(A) synthetase increases 10- to 10,000-fold in various vertebrate tissues and cell lines, exposed to IFN-α, -β, or -γ (1–3, 14). Induction of the (2′-5′)oligo(A) synthetase and activation of RNase F seem to be a central mechanism, although not unique (15), in IFN's antiviral action (1–3, 16) and in the decrease of cell proliferation (17, 18). An elevated level of the enzyme is found in patients receiving IFN therapy (19) or infected by viruses (20). Therefore, it is of considerable interest to elucidate how the level of this enzyme is controlled by IFNs.

In previous work (21–23), we demonstrated that RNA extracted from IFN-treated mouse or human cells and microinjected into *Xenopus laevis* oocytes produces active (2′-5′)oligo(A) synthetase. RNA from untreated cells was inactive. The main (2′-5′)oligo(A) synthetase mRNA activity (E mRNA) behaved as 17S [1.6–1.8 kilobases (kb)] cytoplasmic poly(A)$^+$ RNA, and electrophoresis indicated a main peak and a minor heavier RNA (22, 23). A cDNA library was prepared from 17S RNA of IFN-treated human SV80 cells (simian virus 40-transformed fibroblasts) by cloning in *Escherichia coli* (9), and we report here the identification of a (2′-5′)oligo(A) synthetase E cDNA clone by a positive hybridization-translation method similar to that used for cloning human IFN cDNA (24). The cloned E cDNA hybridizes to RNAs that accumulate in cells after IFN treatment. In SV80 cells, the E cDNA hybridizes to three cytoplasmic RNA species of 1.65, 1.85, and 3.6 kb, whereas in Namalva cells, the predominant RNA is 1.8 kb.

MATERIALS AND METHODS

Cell Cultures and IFNs. Human SV80 cells were grown to confluent monolayers of 5×10^7 cells and lymphoblastoid Namalva cells were grown in suspension to 1.5×10^6 cells per ml as reported earlier (6). Human IFN-β_1 prepared (6, 25) and partially purified (26) to 10^7 units/mg of protein was used to treat cells at 200 units of IFN-β_1 per ml for 12 hr, unless otherwise indicated. IFN-β_1 (>2×10^8 units/mg) purified by monoclonal antibody affinity chromatography and IFN-α were obtained as described (27, 28).

mRNA Isolation and cDNA Cloning. Total cell RNA was prepared as reported earlier (6) with LiCl/urea (29) and purified on oligo(dT)-cellulose (yield, 0.4 mg per 10^9 SV80 cells). The 17S RNA fraction from IFN-treated cells was isolated (unpublished data) in a preparative gel electrophoresis apparatus in 1.5% agarose/6 M urea/25 mM sodium citrate, pH 3.5 (30), and repurified on oligo(dT)-cellulose. ds cDNA was made from 2 μg of 17S RNA as detailed (9, 31). Both strands were made with avian myeloblastosis virus reverse transcriptase (J. Beard) by priming with (oligo)dT, the first strand being tailed with dATP prior to second-strand synthesis (32). Blunt ends were formed on the ds cDNA (9) and the largest fractions were tailed with dCTP and hybridized to dGTP-tailed, *Pst* I-cut pBR322 DNA. *E. coli* MM294 cells (33) were transformed with 20 ng of DNA and 3,600 tetracycline-resistant colonies were isolated, fixed on nitrocellulose (34), and hybridized (35) *in situ* with ^{32}P-labeled cDNA (2×10^8 cpm/μg) prepared from 17S RNA of IFN-treated SV80 cells or from total poly(A)$^+$ RNA of untreated cells.

Hybridization Selection of RNA and Translation. From 250-ml cultures of bacterial clones or pools of 12 clones, plasmid DNA was prepared by CsCl/ethidium bromide banding (36). The DNA, cut with *Eco*RI and heat denatured, was applied (5–30 μg) onto 4-mm disks of nitrocellulose for hybridization to RNA as detailed before (9). Briefly, the filters were preincubated for 2 hr at 37°C in 50% formamide/20 mM Pipes buffer, pH 6.4/0.75 M NaCl/1 mM EDTA (buffer A) and then were incubated with 10 μg of poly(A)$^+$ RNA and 30 μl of buffer A per filter for 16–24 hr at 37°C. Washing conditions (9) were: two times at 37°C with buffer A, four times in 20 mM Tris·HCl, pH 7.5/0.15 M NaCl/1 mM EDTA/0.5% $NaDodSO_4$ (once at 37°C and three times at 52°C), and then four times with 10 mM Tris·HCl, pH 7.5/1 mM EDTA (buffer C) at 52°C. Each filter was next washed alone in buffer C at 52°C, and the RNA was eluted by heating 2 min at 96°C in 0.3 ml of buffer C with 40 μg of rabbit liver tRNA per ml. After quick cooling and ethanol precipitation, the RNA was dissolved in 2 μl of water.

To measure the (2′-5′)oligo(A) synthetase mRNA activity (21),

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Abbreviations: IFN, interferon; ds, double-stranded; kb, kilobase(s).

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0.7 μl of RNA was microinjected into 10 *X. laevis* oocytes (37) and, after 18 hr at 19°C, 0.15 ml of oocyte homogenate (21) was bound to poly(rI):(rC)-agarose beads (P-L Biochemicals) and incubated 16 hr at 30°C with 2.5 mM [α-^{32}P]ATP (0.3 Ci/mmol; 1 Ci = 3.7 × 10^{10} Bq; Amersham) as described (21, 38). The reaction treated with alkaline phosphatase was electrophoresed, and the radioactivity in the (2′-5′)ApA and (2′-5′)ApApA spots was counted (21). Total protein synthesis was measured by [^{35}S]methionine incorporation in rabbit reticulocyte lysates treated with micrococcal nuclease, by using 1 μl of RNA per 12.5-μl reaction (25). The products were analyzed by 12% polyacrylamide gel electrophoresis with $NaDodSO_4$ (25).

mRNA and cDNA Analysis. Poly(A)$^+$ RNA (7 μg) from SV80 or Namalva cells (6) was denatured in 50% formamide/6% formaldehyde, electrophoresed on 1.3% agarose gels in 6% formaldehyde, blotted to nitrocellulose, and hybridized with nick-translated cDNA probes as decribed (6). Nuclease S1 analysis (39) was done as reported earlier (9). Restriction enzymes were from BioLabs and Boehringer Mannheim. DNA fragments separated on agarose (SeaKem) gels were treated by RNase and proteinase K and extracted with phenol/chloroform before end-labeling by polynucleotide kinase or Klenow enzyme and sequence analysis according to Maxam and Gilbert (40).

RESULTS

Identification of a (2′-5′)Oligo(A) Synthetase cDNA Clone. When our cDNA library prepared from IFN-treated SV80 17S RNA was screened by differential hybridization, 8% of the clones hybridized better to [^{32}P]cDNA from 17S RNA of IFN-treated SV80 cells than to cDNA from total RNA of nontreated cells; 40% hybridized to both cDNAs and 10% hybridized only to "nontreated" cDNA (9). The yields of (2′-5′)oligo(A) synthetase from cells (3, 38, 41) suggested that the protein and hence the mRNA are in low concentrations, about 0.03%. Because such rare RNAs may give very weak signals by *in situ* colony hybridization, we decided to use nearly all of the clones of the library for screening by the hybridization-translation method.

Pools of plasmid DNAs on nitrocellulose filters were hybridized to poly(A)$^+$ RNA from IFN-treated cells, and the selected RNAs were assayed for (2′-5′)oligo(A) synthetase mRNA activity in oocytes (Fig. 1*A*). A positive pool or clone should give not only activity over the background but also a substantial enrichment of the (2′-5′)oligo(A) synthetase E mRNA over other mRNAs. Thus, the selected RNAs were also translated in reticulocyte lysates with [^{35}S]methionine (Fig. 1*B*). The ratio of synthetase activity to total protein synthesis (specific activity) was calculated and used during the entire screening procedure (Table 1). For each hybridization, we used 300 μg of poly(A)$^+$ RNA, in which there may be 0.09 μg of E mRNA (0.03%). The cDNA inserts being about one-third of the mRNA length, we used 0.3 μg of insert (3 μg of plasmid DNA) to have a 10-fold excess of each insert over E mRNA. Each 0.4-cm filter was loaded with 12 individual plasmid DNAs. Thirty filters were incubated together and three filters with pBR322 DNA were used as controls. When the RNA eluted from each filter was tested, we found that pool 174 selected the (2′-5′)oligo(A) synthetase mRNA, whereas most of the other 250 pools were negative (Table 1). Each clone of pool 174 was then tested on separate DNA filters in eight different hybridization-translations, three of which are shown in Table 1. In all cases, DNA from clone 174-3 gave a selection of the (2′-5′)oligo(A) synthetase mRNA about 10–50 times higher than the control pBR322 DNA filters. Other cDNA clones sometimes gave values significantly higher than pBR (Fig. 1 and Table 1), but these were false-positive signals. This is based on poor reproducibility and, more important, on the fact that the total translational activity of these DNA-selected RNAs was high and, hence, their specific activity was low (Table 1). In contrast, DNA of clone 174-3 gave consistently a 35- to 100-fold enrichment of the (2′-5′)oligo(A) synthetase mRNA specific activity over pBR322 DNA, allowing its identification as a (2′-5′)oligo(A) synthetase cDNA. This clone E_1 was used to rescreen the 3,600 clones of the library and 1 additional clone (E_2) hybridizing to E_1 cDNA was found.

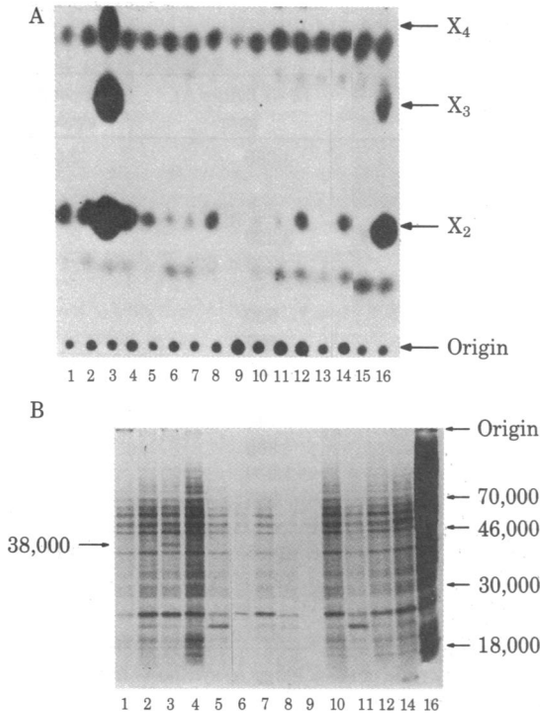


FIG. 1. Hybridization-selection and -translation of (2′-5′)oligo(A) synthetase mRNA. (*A*) Assay of ds RNA-bound (2′-5′)oligo(A) synthetase in extracts of oocytes injected by various RNAs. Autoradiography of the paper electrophoresis at pH 3.5 of the phosphatase-treated [α-^{32}P]ATP-labeled products is shown. X_2, X_3, and X_4 are the positions of $(A2'p)_nA$ dimers, trimers, and tetramer markers. Lanes 1–12, RNA eluted from DNA filters of the 12 cDNA clones forming pool 174; lanes 13 and 14, RNA from pBR322 DNA filters; lane 15, no RNA, water injection; and lane 16, poly(A)$^+$ RNA from IFN-treated SV80 cells. (*B*) Polyacrylamide gel electrophoresis in $NaDodSO_4$ of the [^{35}S]methionine-labeled translation products in reticulocyte lysate of the same RNA samples as in *A* (lane numbers, as in *A*). In the autoradiography, the specific product of clone 174-3 RNA is indicated by an arrow. The background translation is discussed in the text. Molecular weights are indicated.

Structure of the cDNA Insert of Clone E_1. The insert of clone E_1 (174-3) is 675 base pairs long and is flanked by the two reconstituted *Pst* I sites. The major restriction sites are shown in Fig. 2*A*. Clone E_2 was similar to E_1 but slightly smaller. The two strands of the E_1 cDNA insert were subjected to sequence analysis (40) from the restriction sites indicated in Fig. 2*A*. The only long coding frame open was on the strand running counterclockwise to pBR322. The sequence of this strand (Fig. 2*B*) predicts 110 amino acids of the presumed carboxyl-terminal end of the protein. Tyrosine-rich and proline-rich regions are seen. The open frame stops after 330 nucleotides by a TGA codon, followed by several additional stop codons. The 192-nucleotide-long 3′-nontranslated region contains the sequence A-A-U-A-A-A considered as a polyadenylylation signal (42), 14 nucleotides before the start of the poly(A) tail.

Size and IFN Inducibility of the mRNA Hybridizing to Clone E_1. Total cellular poly(A)$^+$ RNA was prepared from SV80 cultures at various times after adding 200 units of IFN-β_1 per ml. We first verified that the E_1 cDNA is colinear with a cell RNA.

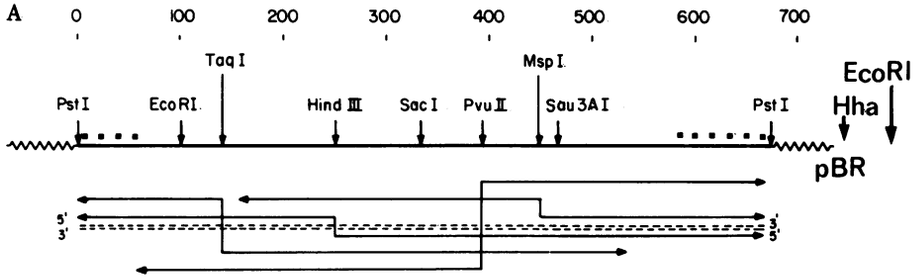
Table 1. Identification by hybridization-translation of the clone of (2′-5′)oligo(A) synthetase cDNA

	Experiment 1		Experiment 2		Experiment 3	
	(2′-5′)Oligo(A), cpm	Specific activity*	(2′-5′)Oligo(A), cpm	Specific activity*	(2′-5′)Oligo(A), cpm	Specific activity*
Total poly(A)$^+$ RNA	4,050	0.007	3,440	0.004	4,900	0.01
RNA selected						
On pBR filters	625–350	0.05	595–570	0.03	725–670	0.02
On plasmid pool 174	2,320		—		—	
On other pools	230 ± 60†		—		—	
Clones of pool 174‡						
174-1	560		700		995	
174-2	625		950	0.04	825	
174-3	*6,460*	*4.78*	*39,800*	*2.7*	*11,235*	*0.75*
174-4	600		1,820	0.48	1,030	0.03
174-5	745		500	0.19	530	
174-6	985		475		630	
174-7	1,270	0.1	365		605	
174-8	395		800	0.02	600	
174-9	490		100		1,030	0.03
174-10	1,465	0.14	290		1,155	0.06
174-11	1,860	0.27	365		735	
174-12	540		320		915	
No RNA	195		185		590	

*Specific activity ratio of (2′-5′)oligo(A) synthesis in mRNA-injected oocytes to translation of same RNA in reticulocyte lysates.
†Average of 28 pools (mean ± SD).
‡Values for E_1 clone 174-3 are shown in italics.

The plasmid DNA was 5′-labeled at the *Eco*RI site of the insert and recut at the *Hha* I site (position 3,925) of pBR322. The fragment (893 bases) contains 487 bases, which should be complementary to the mRNA (Fig. 2). Fig. 3*B* shows that IFN-induced SV80 RNA protects the entire 487-base-long fragment against degradation by nuclease S1. The RNA hybridizing to E_1 cDNA is completely absent from untreated SV80 cells, starts to appear at 4 hr, and is maximal from 8 to 12 hr after IFN treatment. A decrease is seen from 16 to 24 hr. A very similar kinetic pattern was seen for the (2′-5′)oligo(A) synthetase mRNA activity of the same RNA samples measured by injection into oocytes (Fig. 3*A*).

The size of the IFN-treated SV80 RNA detected by the E_1 cDNA probe was studied on electrophoretic blots under dena-



B

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                                        30                                  60
TTT CGG ACG GTC TTG GAA TTA GTC ATA AAC TAC CAG CAA CTC TGC ATC TAC TGG ACA AAG
PHE ARG THR VAL LEU GLU LEU VAL ILE ASN TYR GLN GLN LEU CYS ILE TYR TRP THR LYS
                                        90                                  120
TAT TAT GAC TTT AAA AAC CCC ATT ATT GAA AAG TAC CTG AGA AGG CAG CTC ACG AAA CCC
TYR TYR ASP PHE LYS ASN PRO ILE ILE GLU LYS TYR LEU ARG ARG GLN LEU THR LYS PRO
      Sau3aI                            150                                 180
AGG CCT GTG ATC CTG GAC CCG GCG GAC CCT ACA GGA AAC TTG GGT GGT GGA GAC CCA AAG
ARG PRO VAL ILE LEU ASP PRO ALA ASP PRO THR GLY ASN LEU GLY GLY GLY ASP PRO LYS
                                        210                                 240
GGT TGG AGG CAG CTG GCA CAA GAG GCT GAG GCC TGG CTG AAT TAC CCA TGC TTT AAG AAT
GLY TRP ARG GLN LEU ALA GLN GLU ALA GLU ALA TRP LEU ASN TYR PRO CYS PHE LYS ASN
                                        270                                 300
TGG GAT GGG TCC CCA GTG AGC TCC TGG ATT CTG CTG GTG AGA CCT CCT GCT TCC TCC CTG
TRP ASP GLY SER PRO VAL SER SER TRP ILE LEU LEU VAL ARG PRO PRO ALA SER SER LEU
                                        330                                 360
CCA TTC ATC CCT GCC CCT CTC CAT GAA GCT TGA GAC ATA TAG CTG GAG ACC ATT CTT TCC
PRO PHE ILE PRO ALA PRO LEU HIS GLU ALA
                                        390                                 420
AAA GAA CTT ACC TCT TGC CAA AGG CCA TTT ATA TTC ATA TAG TGA CAG GCT GTG CTC CAT
                                        450                                 480
ATT TTA CAG TCA TTT TGG TCA CAA TCG AGG GTT TCT GGA ATT TTC ACA TCC CTT GTC CAG
EcoRI                                   510
AAT TCA TTC CCC TAA GAG TAA TAA TAA ATA ATC TCT AAC ACC AAA
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FIG. 2. Structure and sequence of E_1 cDNA clone. (*A*) Restriction map of E_1 cDNA. The insert base pairs are numbered in the same direction as pBR322 DNA. The pBR *Eco*RI site is on the right. Both strands of the insert (dashed lines) were subjected to sequence analysis (40) from the restriction sites indicated by the vertical lines. The coding strand runs 5′ to 3′ from right to left. After the right *Pst* I site there were 5′-end tails of 17 G and 72 T (dots), followed by G, A, and the 3 T of the sequence shown in *B*, which are not part of the tails. At the 3′ end, tails of 45 A and 10 C (dots) preceded the left *Pst* I site. The nuclease S1 probe used in Fig. 3 was the complementary strand 5′-labeled at the *Eco*RI site and recut with *Hha* I; in this probe the distance of *Eco*RI to tails is 487 bases. (*B*) Nucleotide sequence with the longest coding frame. The first T is nucleotide 92 following the tails of the insert (right end of *A*).

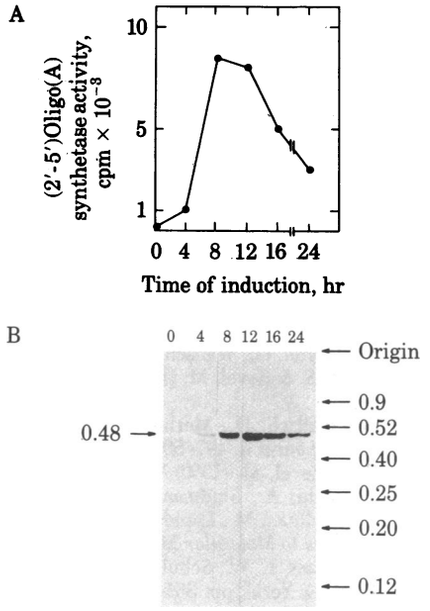

FIG. 3. IFN-dependent induction of RNA colinear to E_1 cDNA clone. (*A*) Poly(A)$^+$ RNA from SV80 cells treated with 200 units of IFN-β_1 per ml for the indicated times was injected into oocytes and the (2'-5')oligo(A) synthetase formed was measured. (*B*) Nuclease S1 analysis (39). Ten micrograms of each RNA as in *A* was hybridized 4 hr at 50°C with 1.5 × 10^4 cpm of the [^{32}P]DNA probe from the E_1 cDNA clone, described in the legend to Fig. 2. After nuclease S1 digestion, the protected DNA fragments were analyzed by electrophoresis on a 6% polyacrylamide gel and the autoradiography is shown. Values are indicated in kb.

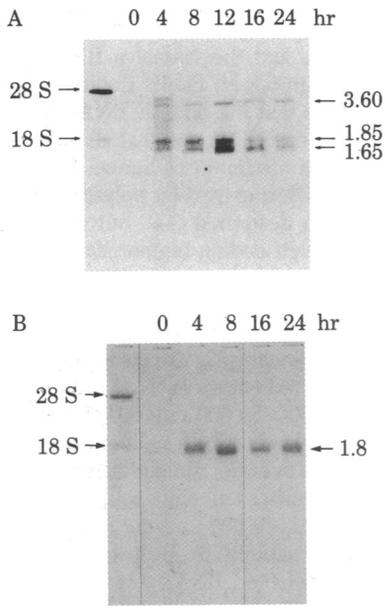

FIG. 4. Size and induction of E_1-specific mRNAs in SV80 and Namalva cells. (*A*) Hybridization of nick-translated [^{32}P]cDNA of clone E_1 to electrophoretic blots of denatured poly(A)$^+$ RNA from SV80 cells (see *Materials and Methods*). The RNAs were prepared at the indicated hour after IFN-β_1 addition. The apparent size of the RNA is indicated on the autoradiography. Left lane, rRNA markers; values are indicated in kb in other lanes. (*B*) Same as *A* with RNA from Namalva cells treated with IFN-α for the indicated time. Left lane, rRNA markers; 1.8 is indicated in kb in other lanes.

turing conditions (Fig. 4*A*). Surprisingly, the E_1 cDNA hybridized to three RNA species, which are induced together after IFN treatment. Two species (about 80% of the total) have apparent lengths of 1.65 and 1.85 kb, and there is a larger RNA of 3.6 kb, which appears coordinately induced. At 4 hr an even larger RNA species is seen but disappears at 8 hr and could be a precursor molecule. Studies with cytoplasmic and polysomal poly(A)$^+$ RNA suggest that the 1.65-, 1.85-, and 3.6-kb RNAs are bound to cytoplasmic ribosomes (not shown). In an experiment with electrophoretically pure IFN-β_1, the same three E_1-specific SV80 RNAs were seen (not shown).

The E_1-specific RNA was also induced by IFN-α in human lymphoblastoid Namalva cells (Fig. 4*B*). In these cells, only one major E_1 RNA was detected at about 1.8 kb. This RNA was in very low concentration in untreated cells and accumulated rapidly from 4 to 8 hr after IFN treatment. Because Namalva RNA directs the synthesis of (2'-5')oligo(A) synthetase in oocytes (22, 23), the small 1.8-kb RNA species appears to encode the enzyme activity. IFN-β_1 induced a similar 1.8-kb RNA in lymphoblastoid cells (not shown).

Translation Product of E_1 cDNA-Selected mRNA. The translation products of the cDNA-selected RNA, labeled by [^{35}S]methionine in reticulocyte lysates are shown in Fig. 1*B*. A clearly identifiable product for E_1 cDNA (174-3) migrates in gel electrophoresis as a M_r 38,000 polypeptide (Fig. 1, lane 3). Larger proteins, such as could be encoded by the 3.6-kb E_1-specific RNA, were not visible in these *in vitro* translation reactions.

DISCUSSION

The objective of this work was to isolate cDNA clones of the IFN-induced (2'-5')oligo(A) synthetase mRNA previously detected by translation in oocytes (21–23). Because we estimated the (2'-5')oligo(A) synthetase mRNA to be rare, we did not rely on *in situ* colony hybridization but screened the cDNA library by positive hybridization-translation. A careful choice of conditions for RNA hybridization-selection was made and the enrichment of (2'-5')oligo(A) synthetase mRNA activity over total mRNA was measured. This allowed us to eliminate many cDNA clones giving false-positive results. One clone, E_1 cDNA (174-3), gave consistently in eight separate experiments a 35- to 100-fold enrichment in specific activity. The (2'-5')oligo(A) synthetase activity produced by oocytes microinjected with E_1 cDNA-selected RNA has the same characteristics as the cellular enzyme (10, 21); it binds to ds RNA and gives the typical (2'-5')oligo(A) nucleotides. Furthermore, the E_1 cDNA clone, which was selected only by hybridization-translation, turned out to represent IFN-induced RNA sequences practically absent from untreated cells and which accumulate after IFN addition with the same kinetics as the (2'-5')oligo(A) synthetase mRNA activity. These kinetics of increase and decrease after IFN are different from those of the HLA mRNAs (6) or of the mRNA for the M_r 56,000 protein (9), which both appear earlier than the E mRNA. Taken together, these results identify the E_1 cDNA clone as corresponding to the (2'-5')oligo(A) synthetase E mRNA.

In SV80 cells, at least three RNAs coordinately induced even by pure IFN-β_1 appear to contain the E_1 cDNA sequence. Two RNAs of approximately 1.65 and 1.85 kb probably correspond to the main 17S E mRNA peak identified by translation in oocytes (22, 23). The larger 3.6-kb RNA also codes for active enzyme and corresponded to a small heavy shoulder of the sucrose gradient E mRNA peak (not shown). Its kinetics of accumulation and its presence on polysomes indicate that it is not a precursor RNA. The relation between the three E RNAs in SV80 cells is intriguing. Hybridization and nuclease S1 analysis shows that they are colinear with the present 0.5-kb E_1 cDNA clone and probably differ in sequences outside this cDNA fragment. Longer E cDNAs were not found in the library. Hybridization to a genomic DNA clone (results not shown) indicated that the three SV80 RNAs hybridize to a common 3.1-kb

*Eco*RI genomic DNA fragment, whereas only the 3.6-kb and 1.85-kb SV80 RNAs and the Namalva RNA hybridize in addition to an upstream 6.8-kb *Eco*RI fragment of this genomic clone. This suggests that the large E RNAs are longer at their 5′ ends than the 1.65-kb RNA and that all the E-specific RNAs hybridize to the same segment of human DNA.

Multiple RNAs differing by their polyadenylylation sites, for example, have been described (43), but the E-specific RNAs may also differ in their coding region. Yang *et al.* (41) found a M_r 100,000 protein in gel electrophoresis of purified HeLa cell (2′-5′)oligo(A) synthetase. The 3.6-kb RNA would be long enough to code for this protein but the 1.65- to 1.85-kb RNAs are too short. In other experiments (22, 23), gel filtration of SV80, HeLa cells, and RNA-injected oocyte extracts indicated two forms of the enzyme, M_rs 60,000–80,000 and 30,000. Therefore, the 1.65- to 1.85-kb RNAs could code for a smaller enzyme. The present work shows that Namalva cells contain mainly one 1.8-kb E RNA and in Namalva and other leukemia cells, gel filtration showed predominantly the M_r 30,000 enzyme (23), which when purified was found fully active (38). By translation in reticulocyte lysate of E cDNA-selected RNA, a M_r 38,000 primary translation product was apparent, which could be further processed in the cells; the M_r 100,000 protein was not seen but large products are sometimes difficult to detect by *in vitro* translation. The structure and relationship between the different enzyme forms remains to be established, but our results are in line with their being different translation products of one or more E genes.

The cDNA clones for an IFN-induced (2′-5′)oligo(A) synthetase mRNA offer a tool for studying the molecular mechanism of IFN action. Variations in enzyme level are found in cells undergoing changes in differentiation or growth rate (14, 44–46), although IFN appears as the main regulator of the enzyme level (1–3, 46). Leukocytes of patients with viral diseases contain elevated levels of the (2′-5′)oligo(A) synthetase (20), whereas in some leukemias decreased levels are seen (47). The cloned E_1 cDNA can now be used to measure the amount of specific mRNAs and the activity of the (2′-5′)oligo(A) synthetase gene(s). In particular, it will be interesting to determine if the multiple E mRNA species are always coordinately regulated or mediate different effects of the complex IFN system.

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